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Evaluation of Methyl Iodide as a Soil Fumigant in Container and Small Field Plot Studies

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Abstract: Methyl iodide was evaluated as a soil fumigant as a potential replacement for the widely used soil fumigant methyl bromide. In container trials, methyl iodide was significantly more effective than methyl bromide against the plant parasitic nematodes *Meloidogyne incognita*, *Heterodera schachtii* and *Tylenchulus semipenetrans* and the plant pathogenic fungus *Rhizoctonia solani*. In small field plots, soil populations of root-knot nematodes were no longer detected after methyl iodide fumigation at an application rate of 112 kg ha^{-1}. However, after growing a susceptible lima bean host for two months, substantial root-knot galling occurred, while *Rhizobium* nodulation was absent. At 168 kg ha^{-1} of methyl iodide, root-knot galling was reduced to less than 1%, and no *Pythium* propagules were recovered on selective detection media. These efficacy data support the conclusion that methyl iodide is a likely candidate for replacing methyl bromide as a soil fumigant. © 1998 SCI.

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Key words: methyl bromide; methyl iodide; soil fumigation; *Meloidogyne incognita*; *Heterodera schachtii*; *Tylenchulus semipenetrans*; *Rhizoctonia solani*; *Pythium* spp.

1 INTRODUCTION

Since the late 1950s, methyl bromide (CH_3Br), alone and in combination with chloropicrin, has been widely used internationally as a broad-spectrum soil fumigant to control plant parasitic nematodes, pathogens, weeds and soil arthropods. For most soil fumigation purposes, CH_3Br is injected into the soil 20–30 cm deep at 224–448 kg ha^{-1}. In addition, CH_3Br is used for post-harvest, commodity quarantine fumigation and as a structural fumigant.[1] Due to its listing under the Clean Air Act as a class I ozone-depleting substance, CH_3Br production and importation are scheduled to be banned in the USA by January 1, 2001. The impact of the withdrawal of CH_3Br as a soil fumigant will depend on the availability of effective alternative pest management tools. None of the currently available pesticide, cultural or biological control options has the efficacy and wide spectrum of CH_3Br.[2] We recently reported preliminary results with methyl iodide (CH_3I) which, at equivalent molar rates, was as effective as, or more effective than, CH_3Br against a wide range of fungal pathogens, nematodes and weeds.[3] Although CH_3I was tested in the past as a fumigant against insect pests of stored products,[4–6] it has not been definitively evaluated as a potential soil fumigant. UV exposure causes rapid photolysis of CH_3I that makes its participation in stratospheric ozone destruction unlikely.[7,8]

The objective of this research was to compare the dose response of CH_3Br with CH_3I against three different plant parasitic nematodes and a fungal plant pathogen in container studies. A preliminary report was

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published earlier.[9] We also report on dose-effect studies with CH_3I against plant parasitic nematodes and *Pythium* spp. in small field plot trials.

2 MATERIAL AND METHODS

2.1 Test inoculum

Root-knot nematodes were obtained from two- to three-month-old tomato plants (*Lycopersicon esculentum* Mill. cv. Tropic) that had been inoculated with *Meloidogyne incognita* (Kofoid & White) Chitwood six weeks after seeding. Nematode eggs were collected by a modified extraction technique.[10] Briefly, galled roots were washed free of soil under running water and cut into 2-cm-long pieces. These were triturated for two 30-s intervals at maximum speed with a blender (Waring, New Hartford, CT) in a sodium hypochlorite solution (2·6 g litre^{-1}). The eggs were collected on a 30-μm-pore sieve, washed carefully, and placed on Baermann funnels for two days. Freshly hatched juveniles (approximately 80 000) were collected and carefully mixed into pasteurized sandy soil (2000 cm^3). This soil (93% sand, 4% silt, 3% clay; 0% organic content; pH 7·5) was used in all experiments unless stated otherwise. Mature cysts of *Heterodera schachtii* Schmidt were obtained from three-month-old greenhouse-grown sugar beets infested with the sugar beet cyst nematode. The cysts were elutriated and collected onto sieves. Cysts (approximately 8000) were thoroughly mixed into pasteurized sandy soil (2000 cm^3). Rhizosphere samples were taken from a citrus orchard at the University of California Riverside Citrus Research Center. The particular location was known to be heavily infested with *Tylenchulus semipenetrans* Cobb. Citrus feeder roots were carefully washed out and cut into 1-cm-long pieces. The root pieces (56 g) were mixed into pasteurized sandy soil (2000 cm^3). *Rhizoctonia solani* Kühn AG4 was grown on sterilized millet seeds for three weeks. The inoculum was then air dried and stored in glass bottles at 4°C until use. Three sets of small muslin bags (Hubco Soil Sample Bags, Forestry Supplier, Inc., Jackson, MS) were filled with soil (50 cm^3) infested with *M. incognita*, *H. schachtii* or *T. semipenetrans*. A fourth set received pasteurized soil (50 cm^3) into which 20 *Rhizoctonia*-infested millet seeds were mixed.

2.2 Container trials

Tests were conducted during the winter months 1994/1995 at the University of California Riverside Agricultural Operations. White plastic containers, 28 cm wide and 38 cm deep and filled with sandy soil (18 000 cm^3), served as experimental units. Holes in the bottom allowed drainage and aeration. All trials were designed as randomized complete blocks with four replicates per treatment. Four muslin bags, each filled with soil infested with a different nematode species or *R. solani*, were placed onto a soil layer (10 cm deep) in the bottom of each container. They were covered with a second layer (20 cm deep) of the same soil. Soil moisture in all trials was approximately −10 bar. Soil temperature at the time of treatments and during the time the containers remained covered was between 15°C and 24°C. CH_3Br (Great Lakes Chemical Corporation, West Lafayette, IN) was cooled to −56°C and CH_3I (RSA Corporation, Danbury, CT) was kept at 4°C to allow safe handling. The required amounts (5·9, 11·8, 17·8, 23·6, 29·5, 44·3, 88·6, 177·2 and 354·4 mmol m^{-2}) were pipetted into cooled 5-ml glass vials and sealed air-tight. CH_3Br vials were transported on dry ice to the trial site, CH_3I was kept cool on regular ice. The vials were placed onto the soil surface in the appropriate container and opened. The containers were immediately covered with black polyethylene (0·1 mm thick) which was secured over the top of the container with a large rubber band. The polyethylene was removed four days later and the containers were allowed to aerate for another day. Sample bags with nematode inoculum were placed on Baermann funnels and the number of nematode juveniles was determined five days later. Cyst nematode hatch was supported by zinc chloride solution (3 mM).[11] The extraction efficacy for *M. incognita* was approximately 34%. Non-treated samples with *H. schachtii* yielded about 1400 larvae per 50 cm^3 while approximately 2500 larvae per 50 cm^3 were recovered from the citrus nematode-infested soil. *Rhizoctonia*-infested millet seeds were recovered by soil sieving and placed on water agar. Viability of the fungus was evaluated after three days incubation at 23°C.

2.3 Micro-plot trials

Two field trials were conducted at the University of California South Coast Research and Extension Center at Irvine, CA. Soil type was a San Emigdio sandy loam: 75·4% sand, 12·5% silt, 12% clay; 0·45% organic matter; pH 7·2. The first trial was conducted in June–July 1995. The site was heavily infested with *M. incognita* because it had been planted the previous year to root-knot nematode-susceptible tomatoes. The heavily galled roots were not removed but remained in the ground to cause a strong infestation pressure. The soil was naturally infested with *Pythium* spp. Pringsheim. The individual beds (1 × 1 m) were installed on 1·5 m centers. Low-volume irrigation tubing with emitters (2 litre h^{-1}) spaced at 0·3 m intervals was placed on top of the beds to moisten the soil before application of CH_3I and to provide water for the following crop. Soil moisture at the time of the treatments was approximately −10 bar. The individual beds were covered by hand

with high density transparent polyethylene (0·025 mm thick) with the edges buried and secured by additional soil. CH_3I (28, 56, 112, 168, 224 kg ha^{-1}) was applied by inserting glass pipettes through the polyethylene and 0·05 m into the soil. One quarter of the total amount was applied at four spots equidistant from each other and the edges. The holes in the polyethylene were immediately sealed with self-adhesive tape. The control treatment received no fumigant. All covers were removed four days later. One week later, six soil cores (25 cm) were taken from each bed, thoroughly mixed and pooled. Subsamples (50 ml) were placed on Baermann funnels for root-knot nematode extraction. Propagules of *Pythium* spp were determined by soil processing and dilution plating on selective MPVM agar.[12,13] Lima beans (*Phaseolus lunatus* L. cv. Henderson) were seeded in two rows per bed, separated in the middle by the irrigation line. These plants served as indicators for reappearing root-knot nematodes from zones the fumigant did not penetrate or where lethal concentrations were not achieved. They also helped to evaluate the effect of CH_3I on naturally occurring *Rhizobium* symbionts. Two months later, ten randomly selected plants were removed from each bed and their root system was rated for root-knot nematode galling (scale 0–100). Occurrence of *Rhizobium* nodules was registered as present or not. A second trial was conducted in August 1995 in a field adjacent to the first. This field had been fallow for several years and was not infested with plant parasitic nematodes. Effects of the CH_3I treatments on nematodes were determined by burying small muslin bags containing field soil (50 cm^3) with roots heavily infested with the citrus nematode (*T. semipenetrans*). In each bed one bag was buried (0·3 m deep) and recovered one day after polyethylene removal. The same extraction methodology was employed as with the previously described container trials. Soil sampling and processing for *Pythium* propagules was also conducted as described before. Soil moisture at the time of treatments was approximately −10 bar.

2.4 Statistical analysis

All data were subjected to analysis of variance. Means of significant treatment effects were separated by Fisher's protected least significant difference test at $P = 0{\cdot}05$.

3 RESULTS

3.1 Field container trials

CH_3I was consistently more effective than CH_3Br against any of the tested nematode species and the plant pathogenic fungus *R. solani* (Table 1). At the lowest rate tested CH_3Br significantly reduced the population of citrus nematodes and root-knot nematodes but had little effect on sugar beet cyst nematodes. In contrast, the same rate of CH_3I reduced the populations of all three species dramatically. By doubling the rate, CH_3I eliminated all nematodes while 10–76% of the nematode populations survived the CH_3Br treatment. Almost a third of the *H. schachtii* population survived 23·6 mmol m^{-2} CH_3Br. The soilborne fungus *R. solani* was much less sensitive to both fumigants than were the

TABLE 1
Comparison of Efficacy of Methyl Bromide and Methyl Iodide against Three Plant Parasitic Nematodes and a Soilborne Fungal Pathogen in Field Container Trials[a]

	Meloidogyne incognita		Heterodera schachtii		Tylenchulus semipenetrans		Rhizoctonia solani	
mmol m^{-2}	CH_3Br	CH_3I	CH_3Br	CH_3I	CH_3Br	CH_3I	CH_3Br	CH_3I
0·0	100d		100c		100c		100d	
5·9	66·6c	22·5b	85·7c	54·8b	80·3b	5·1a	NT[b]	NT
11·8	10·7b	0·0a	75·9bc	0·0a	42·2a	0·2a	NT	NT
17·8	3·8ab	0·0a	35·8ab	0·0a	17·4a	0·1a	NT	NT
23·6	0·5a	0·0a	30·6ab	0·0a	4·9a	0·0a	NT	NT
29·5	0·0a	0·0a	4·3a	0·0a	0·2a	0·0a	100·0d	100·0d
44·3	NT	NT	NT	NT	NT	NT	100·0d	90·0cd
88·6	NT	NT	NT	NT	NT	NT	92·5cd	27·5c
177·2	NT	NT	NT	NT	NT	NT	77·5c	7·5b
354·4	NT	NT	NT	NT	NT	NT	10·0ab	0·0a

[a] Data are means of a representative trial with four replicates. Statistical analysis was conducted on original data but presented as percentage recovery of second stage nematode juveniles and percentage survival of *Rhizoctonia solani* from infested millet seed. Mean comparisons among treatments were calculated separately for each species. Means followed by the same letters are not significantly different using Fisher's Protected LSD ($P = 0{\cdot}05$).
[b] Not tested.

nematode species. While even at the highest rate tested, 10% survival was observed after CH_3Br fumigation, the fungus was dramatically reduced with CH_3I at 88·6 mmol m^{-2} and eliminated at 354·4 mmol m^{-2}.

3.2 Small field plots

As little as 28 kg ha^{-1} CH_3I significantly reduced root-knot juvenile population in the soil. However, this was not sufficient to suppress root galling on lima beans examined two months later (Fig. 1). Only when the population of *M. incognita* was reduced at the beginning of the growing season to less than 1% was there a significant impact on root galling. At 168 kg ha^{-1} no living root-knot juveniles were recovered in post-planting samples and root-knot galling after two months was suppressed to less than 1% (Fig. 1). In the second trial, no citrus nematodes were recovered from samples taken from plots fumigated with 56 kg ha^{-1} CH_3I. *Pythium* spp. were not detected in treatments

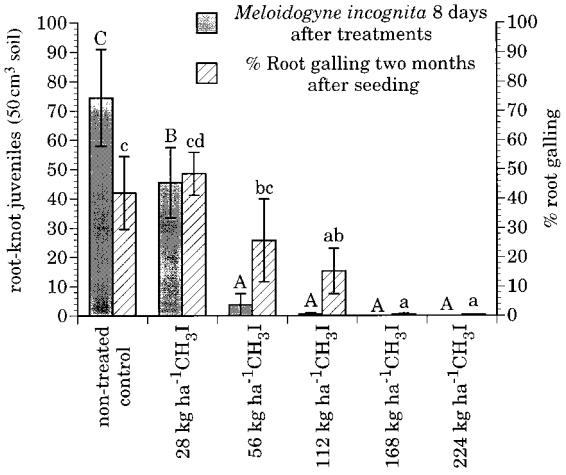

Fig. 1. Effect of soil fumigation with methyl iodide on *Meloidogyne incognita* population and root galling of Lima beans. Bars represent standard error of the mean. Means followed by the same letters were not significantly different according to Fisher's Protected LSD ($P = 0{\cdot}05$).

receiving 168 kg ha^{-1} CH_3I or more (Table 2). No *Rhizobium* nodules were found on two-month-old Lima bean roots grown in soil which had received in excess of 112 kg ha^{-1} (data not shown).

4 DISCUSSION AND CONCLUSIONS

Under the described test conditions and equivalent molar rates, CH_3I was significantly more active against all three nematodes species and *R. solani* than CH_3Br. The container studies and small-plot field data support our previous reports that CH_3I is effective against a wide range of organisms, including plant parasitic nematodes and plant pathogenic fungi. Considering the high nematode infestation and non-optimized application technique, CH_3I was very effective in the field trials at similar rates to those previously reported for CH_3Br.[14]

The broad spectrum of activity of CH_3Br is attributed to the methylation of various functional groups in proteins, peptides and nucleic acids.[15] Although the mode of action of CH_3I is likely to be similar, the superior efficacy may be due to relative reactivity or the retention time of the fumigants in the test system. Under current commercial application methods, a considerable proportion of the CH_3Br applied diffuses through the cover and into the atmosphere within a few hours.[16–18] CH_3I has only a quarter of the vapour pressure of CH_3Br and may therefore be less prone to fast escape. It has been predicted that eventually more CH_3I will volatilize into the air due to slower soil degradation.[8] However, unlike the UV-stable CH_3Br, this carries no consequences for impact on the ozone layer. Since CH_3I is broken down rapidly by UV radiation, it is estimated to have a relatively short atmospheric residence time of four to eight days.[19] Intensive studies are needed to evaluate safety and environmental behavior of CH_3I.

We did not specifically address potential differences in sensitivity of various nematode life stages to CH_3I.

TABLE 2
Efficacy of Soil Fumigation with Methyl Iodide against Citrus Nematodes and *Pythium* spp. in a Small Plot Field Trial[a]

Dosage		*Tylenchulus semipenetrans*	*Pythium* spp.
CH_3I (kg ha^{-1})	*m mole m^{-2}*	*Second-stage juveniles 50 cm^{-3}*	*Propagules g^{-1} soil*
0	0	399 (±58)b	228 (±46)c
28	19·7	14 (±5)a	116 (±26)b
56	39·5	0a	56 (±33)ab
112	78·9	0a	25 (±11)a
168	118·3	0a	0a
224	157.8	0a	0a

[a] Mean comparisons among treatments were calculated separately for each species. Means followed by the same letters are not significantly different using Fisher's Protected LSD ($P = 0{\cdot}05$).

However, the nature of our test material provides preliminary evidence that there was very little difference. *M. incognita* were exposed to the fumigants as second-stage juveniles while the *T. semipenetrans* inoculum was mainly present as eggs. *H. schachtii* was included in the test as mature cysts containing eggs. Although *T. semipenetrans* seemed to be the most sensitive test organism, the differences in survival among the three nematode species were minor. This is in contrast to the nematode recovery after CH_3Br fumigation, which differed notably among the species. *H. schachtii* was able to withstand considerably higher rates than the other two species. Differences in toxicity of CH_3Br to various plant parasitic nematodes were reported earlier.[20] In our trials, even the highest rate of CH_3Br tested did not completely eliminate the sugar beet cyst nematode.

The container and field trials demonstrated that soil-dwelling life stages of nematodes are generally the organisms most sensitive to CH_3I. Similar results with various other fumigants were previously reported.[14] The authors also pointed out that between 9 and 15 times higher rates of the fumigants were required to eliminate plant parasitic nematodes and pathogenic fungi under field conditions. This is due to the confined conditions in container trials which allow a more precise estimation of the fumigant concentration and its retention in the soil volume. It also eliminates invasion and recolonization by nematodes and fungi from infested but sub-lethally treated soil areas. In this respect it remains to be investigated whether endoparasitic nematodes can survive soil fumigation within plant tissues. CH_3Br has the ability to penetrate such tissues and provide control which is not achieved with many of the other nematicides. Similarly, soilborne fungi which produce resilient resting or survival structures such as sclerotia or chlamydospores, especially when occurring in crop residues, may require considerably higher application rates.

In conclusion, the container trials confirmed the excellent performance of CH_3I as a soil fumigant. Our studies indicate that its efficacy is significantly better than that of CH_3Br at equal molar rates. The small field-plot studies suggested that the application rate under commercial production conditions might be similar to that of CH_3Br. The insignificant ozone depletion potential of CH_3I and its excellent efficacy against soil pests and pathogens make it a strong candidate to replace CH_3Br. However, additional toxicological and ecological studies are needed to support the development of CH_3I for production agriculture.

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